

Claims

1. A preparation comprising *Bifidobacterium breve* and a mixture of at least two non-digestible soluble carbohydrate components A and B, the carbohydrate component A being present in an amount of from 5 to 95 % by weight of the sum of carbohydrate components A and B, at least 50% of the total non-digestible soluble carbohydrates being selected from disaccharides to eicosasaccharides, components A and B differing either
(i) in the (average) number of monosaccharide units of the carbohydrate, component A having an average chain length which is at least 5 monosaccharide units lower than the average chain length of component B, or
(ii) in the structure of the monosaccharide units of the carbohydrate, or
(iii) both.
2. A preparation according to claim 1, wherein the carbohydrate component A has a different structure from the carbohydrate component B.
3. A preparation according to claim 1 or 2, wherein the carbohydrate components A and B differ in the (average) number of monosaccharides units, component A being selected from indigestible monosaccharides up to hexasaccharides of the same carbohydrate structure, and component B being selected from indigestible heptasaccharides and higher polysaccharides of the same carbohydrate structure.
4. A preparation according to any one of Claims 1-3, wherein the carbohydrate component A comprises 95 to 60 wt% and the carbohydrate B comprises 5 to 40 wt%, with $A + B = 100$ wt%.
5. A preparation according to any one of claims 1-4, wherein at least 60 wt%, preferably 80 to 100 wt% of carbohydrate components A belong to the group of galacto-oligosaccharides.
6. A preparation according to any one of claims 1-5, wherein at least 60 wt%, preferably 80 to 100 wt% of carbohydrate components B belong to the group of fructo-polysaccharides, including inulin.
7. A preparation according to any one of claims 1-6, comprising 10^7 to 10^{11} cfu, preferably 10^8 to 10^{10} cfu of *Bifidobacterium breve* per g of total non-digestible soluble carbohydrate.

8. A preparation according to any one of claims 1-7 for use as a supplement, wherein the probiotic *Bifidobacterium breve* is present in the supplement in an amount of from 1×10^6 to 1.5×10^{11} cfu/g, preferably from 3×10^7 to 5×10^{10} cfu/g, more preferably from 5×10^8 to 1×10^{10} cfu/g, calculated on the basis of the supplement.
9. A preparation according to any one of claims 1-7 for use as an infant nutrition, wherein the *Bifidobacterium breve* is present in the supplement in an amount of from 1×10^4 to 1×10^{10} cfu/g, preferably from 5×10^6 to 3×10^9 cfu/g, more preferably from 1×10^7 to 5×10^8 cfu/g of the infant supplement.
10. An infant nutrition supplement comprising a preparation according to any one of claims 1-8, and further comprising digestible carbohydrate, a lipid source, a protein source, or a mixture thereof.
11. An infant nutrition comprising a preparation according to any one of claims 1-7 and 9, and further comprising digestible carbohydrate, a lipid source, and a protein source.
12. Use of a preparation according to any one of claims 1-9 for the manufacture of a composition for the normalisation of the *Bifidobacterium* species composition in the gastro-intestinal tract of non- or partially breast-fed infants to the composition in breast-fed infants.
13. Use of a preparation according to any one of claims 1-9 for the manufacture of a composition for the prevention or treatment of one or more immune disorders.
14. Use according to claim 13, wherein said immune disorders are selected from allergy, atopy, allergic rhinitis, food hypersensitivity, atopic dermatitis, eczema and asthma.
15. Use according to claim 13 or 14, wherein said immune disorders are selected from diarrhoea and viral diarrhoea.
16. Use of a preparation according to any one of claims 1-9 for the manufacture of a composition for preventing and/or treating energy malabsorption.
17. Use of a preparation according to any one of claims 1-9 for the manufacture of a composition for inhibiting the infiltration of eosinophils, neutrophils and mononuclear

cells in allergic lesions, inhibiting the Th2 type immune response and/or stimulating the Th1 mediated immune response.

18. Use of a mixture of at least two non-digestible soluble carbohydrate components A and B, the carbohydrate component A being present in an amount of from 5 to 95 % by weight of the sum of carbohydrate components A and B, at least 50% of the total non-digestible soluble carbohydrates being selected from disaccharides to eicosasaccharides, components A and B differing either in the (average) number of monosaccharide units of the carbohydrate, or in the structure of the monosaccharide units of the carbohydrate, or both, for decreasing the relative amounts of *Bifidobacterium catenulatum*, *B. pseudocatenulatum* and/or *B. adolescentis* in the gastro-intestinal tract of non- or partially breast-fed infants.

19. Oligonucleotides comprising SEQ ID selected from SEQ ID No 1, SEQ ID No 2, SEQ ID No 4, SEQ ID No 5, SEQ ID No 7, SEQ ID No 8, SEQ ID No 10, SEQ ID No 11, SEQ ID No 13, SEQ ID No 14, SEQ ID No 16, SEQ ID No 17, SEQ ID No 19, SEQ ID No 20, SEQ ID No 22, SEQ ID No 23, SEQ ID No 25, SEQ ID No 26, and sequences complementary thereto.

20. Oligonucleotide probe for detection of a nucleic acid target sequence which is characteristic of the species of the genus *Bifidobacterium*, said probe being selected from:

- 1) a labelled oligonucleotide which specifically hybridises to *B. adolescentis* DNA represented by SEQ ID No 3 or a sequence complementary thereto;
- 2) a labelled oligonucleotide which specifically hybridises to *B. angulatum* DNA represented by SEQ ID No 6 or a sequence complementary thereto;
- 3) a labelled oligonucleotide which specifically hybridises to *B. bifidum* DNA represented by SEQ ID No 9 or a sequence complementary thereto;
- 4) a labelled oligonucleotide which specifically hybridises to *B. breve* DNA represented by SEQ ID No 12 or a sequence complementary thereto;
- 5) a labelled oligonucleotide which specifically hybridises to *B. catenulatum* DNA represented by SEQ ID No 15 or a sequence complementary thereto;
- 6) a labelled oligonucleotide which specifically hybridises to *B. dentium* DNA represented by SEQ ID No 18 or a sequence complementary thereto;
- 7) a labelled oligonucleotide which specifically hybridises to *B. infantis* DNA represented by SEQ ID No 21 or a sequence complementary thereto;
- 8) a labelled oligonucleotide which specifically hybridises to *B. longum* DNA represented by SEQ ID No 24 or a sequence complementary thereto;
- 9) a labelled oligonucleotide which specifically hybridises to all *Bifidobacterium* DNA represented by SEQ ID No 27 or a sequence complementary thereto.

21. A method of species-specifically detecting species of the genus *Bifidobacterium* found in human, particularly human infants, comprising the steps of:

(A) contacting a sample with an oligonucleotide probe in a hybridising solution, wherein said probe is selected from the group consisting of:

- 1) a labelled oligonucleotide which specifically hybridises to *B. adolescentis* DNA represented by SEQ ID No 3 or a sequence complementary thereto;
- 2) a labelled oligonucleotide which specifically hybridises to *B. angulatum* DNA represented by SEQ ID No 6 or a sequence complementary thereto;
- 3) a labelled oligonucleotide which specifically hybridises to *B. bifidum* DNA represented by SEQ ID No 9 or a sequence complementary thereto;
- 4) a labelled oligonucleotide which specifically hybridises to *B. breve* DNA represented by SEQ ID No 12 or a sequence complementary thereto;
- 5) a labelled oligonucleotide which specifically hybridises to *B. catenulatum* DNA represented by SEQ ID No 15 or a sequence complementary thereto;
- 6) a labelled oligonucleotide which specifically hybridises to *B. dentium* DNA represented by SEQ ID No 18 or a sequence complementary thereto;
- 7) a labelled oligonucleotide which specifically hybridises to *B. infantis* DNA represented by SEQ ID No 21 or a sequence complementary thereto;
- 8) a labelled oligonucleotide which specifically hybridises to *B. longum* DNA represented by SEQ ID No 24 or a sequence complementary thereto;
- 9) a labelled oligonucleotide which specifically hybridises to all *Bifidobacterium* DNA represented by SEQ ID No 27 or a sequence complementary thereto, and

(B) determining whether said probe hybridises to nucleic acids in said sample so as to detect whether said species of said genus is present in said sample.

22. A method of species-specifically quantifying the detected species of the genus *Bifidobacterium* found in humans, particularly in human faeces, according to claim 21, wherein said quantifying method is real time PCR.

23. A method of species-specifically detecting species of the genus *Bifidobacterium* found in human, particularly human infants, comprising the steps of:

- a) performing a nucleic acid sequence amplification procedure using a primer set comprising the oligonucleotide primer of claim 19, preferably the oligonucleotide primer of SEQ ID No selected from SEQ ID No 1, SEQ ID No 4, SEQ ID No 7, SEQ ID No 10, SEQ ID No 13, SEQ ID No 16, SEQ ID No 19, SEQ ID No 22, SEQ ID No 25, and respectively with the oligonucleotide primer of SEQ ID selected from SEQ ID No 2, SEQ ID No 5, SEQ ID No 8, SEQ ID No 11, SEQ ID No 14, SEQ ID No 17, SEQ ID No 20, SEQ ID No 23, SEQ ID No 26; and

b) determining whether the oligonucleotide probe above mentioned hybridises to the nucleic acid target sequence.

24. A diagnostic kit for the detection in a sample of *Bifidobacterium* species selected from *Bifidobacterium adolescentis*, *B. angulatum*, *B. bifidum*, *B. breve*, *B. catenulatum*, *B. dentium*, *B. infantis* and *B. longum*, by means of hybridisation analysis, comprising at least a DNA probe according to claim 20 as well as one or more further means required for hybridisation analysis, such as denaturation liquid, a hybridisation liquid, a washing liquid, a solid carrier, a hybridisation vessel and label detecting means.

25. A diagnostic kit for the detection in a sample of *Bifidobacterium* species selected from *Bifidobacterium adolescentis*, *B. angulatum*, *B. bifidum*, *B. breve*, *B. catenulatum*, *B. dentium*, *B. infantis* and *B. longum*, by means of PCR analysis, comprising a set of DNA primers according to claim 19 as well as one or more further means required for PCR analysis, such as a polymerase, a polymerisation liquid, an oil overlay, a reaction vessel and means for detecting the amplified DNA.